HU, Sol fiber cross-sectional area, as an index of atrophy, is reduced in both WT and PTN. In basal conditions, PTN overexpression increases type I and decreases type IIA fibers, suggesting that Sol becomes slower. HU has no effect on Sol fiber typing in transgenic mice, suggesting that PTN may counteract the HU-induced slow-to-fast shift. HU increases the resting chloride conductance of sarcolemma by 36% in WT mice and only by 17% in PTN mice, as expected from phenotype shift. HU increases gene expression of ClC-1 chloride and Nav1.4 sodium channels, and decreases expression of Ca^{2+}-activated K^+ channels (realtime-PCR), in both WT and PTN mice. Although expression of ATP-sensitive K^+ channels subunits is similar in WT and PTN Sol in basal conditions, HU increases their expression only in PTN mice. In parallel, the resting potassium conductance of Sol fibers is increased after HU only in PTN mice. Expression of the proangiogenic genes, VEGF-A and KDR, is decreased after HU in WT mice. Surprisingly, PTN overexpression also decreases expression of VEGF-A and KDR in basal conditions, so that no further effect of HU is observed in PTN mice. The results suggest that PTN overexpression may modify expression and function of chloride and potassium channels, thereby modulating the adaptation of soleus muscle to disuse (supported by Italian Space Agency).

Cardiac Muscle I

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Modeling Ca^{2+} Induced Ca^{2+} Release Between Neighboring Ryanodine Receptors

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In the heart, Ca^{2+} is released from the sarcoplasmic reticulum (SR) through ryanodine receptors (RyR). A small Ca^{2+} trigger activates a RyR, which then mediates a larger Ca^{2+} flux sufficient to activate neighboring RyRs. The inherent positive feedback of Ca^{2+} induced Ca^{2+} release (CICR) should cause Ca^{2+} release to continue until the SR is empty. This phenomenon does not occur in cells. In order to understand why, we have developed a simple physical model to describe CICR using simulations of discrete RyR channels on a two dimensional grid. RyR open probability (P_o) is defined through a traditional two-site Hill function comprised of one cytosolic Ca^{2+} activation and one cytosolic Ca^{2+} inactivation sites per subunit. The diffusion equation for a steady state point source of Ca^{2+} current defines the Ca^{2+} concentration everywhere in the system. Single channel experimental Ca^{2+} dissociation constant values were used. Our Metropolis Monte Carlo simulations quantitatively reproduce P_o's measured as a function of cytosolic Ca^{2+} for both single RyR as well as a two-RyR channel system reconstituted in bilayers. Our simulations suggest a mechanism for CICR termination requiring only a reduction in unitary Ca^{2+} current. Small changes in Ca^{2+} current produce large and sudden changes in overall activity in RyR arrays. Our simulations show reducing current may terminate release well before depletion of the SR at Ca^{2+} loads consistent with experiment. This current-termination occurs independently from any form of luminal regulation or coupled gating. Thus, we propose CICR termination may be due to a drop in single channel Ca^{2+} current as local intra-SR Ca^{2+} levels fall during release.

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In Vivo Simvastatin Treatment Differentially Affects Caveolin-1 and Caveolin-3 Expression in the Adult Rat Myocardium

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Drugs such as statins, which modify cholesterol synthesis and transport, have the capacity to alter the expression of many proteins through transcriptional regulation *via* sterol regulatory elements (SRE) in their promoters. One group of such proteins are the caveolins, structural and regulatory elements of caveolae. Caveolae regulate diverse processes including $[Ca^{2+}]_i$ handling and adrenergic responsiveness in the adult cardiac myocyte [1]. Given the cholesterol- and caveolin-dependence of caveolae, we hypothesised that statin treatment would affect myocyte caveolae with consequences for contractile function. Male Wistar rats were treated with 40 mg/kg simvastatin for 2 weeks. Serum cholesterol was significantly reduced following statin treatment (from 554 to 460 μg/ml, n=11; $P<0.01$, Student's t-test), and this was associated with a corresponding 23% reduction in myocardial cholesterol (n=6, $P<0.05$). Caveolin 1 expression was attenuated by 84% in the statin-treated group (n=3, $P<0.001$), but surprisingly this was associated with a 404% increase in caveolin 3 expression (n=6, $P<0.05$). Changes in caveolin expression were accompanied by a significant decrease in time to half relaxation and time to half decay of the $[Ca^{2+}]_i$ transient in field-stimulated isolated ventricular myocytes (n=13-20 cells from 3-4 hearts; $P<0.05$). These data show, for the first time, that *in vivo* simvastatin treatment influences the profile of caveolin expression in the myocardium and increases lusitropy. This represents a novel pleiotropic effect of statins which would be predicted to improve diastolic filling of the heart *in situ*.

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[1] Calaghan S, White E. Caveolae modulate excitation-contraction coupling and beta2-adrenergic signalling in adult rat ventricular myocytes. Cardiovasc Res 2006;69:816-24.

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NAADP is a Physiologically-Relevant Calcium Mobilising Compound in Atrial Myocytes

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NAADP is a calcium mobilising compound acting via a two-pool mechanism (1). In both ventricular and atrial myocytes, NAADP increases cellular calcium transients through enhancement of SR calcium load (2,3).

In ventricular myocytes Ned-19, a non-competitive antagonist of the NAADP receptor, abolishes the contractile response to NAADP(4). Furthermore, both bafilomycin and Ned-19 decrease whole-cell calcium transients and, after Ned-19 exposure, bafilomycin has no further effect (4).

Tissue level of NAADP is raised after isoprenaline perfusion in whole heart, consistent with a role for NAADP in the beta-adrenergic response (2).

This project aimed to measure the response of atrial myocytes to NAADP and investigate the relevance of the pathway to beta-adrenergic signalling.

Enzymatically-isolated guinea pig atrial myocytes were loaded with fluo-5F calcium indicator dye and NPE-caged NAADP (5μM). Cells were stimulated during superfusion with physiological salt solution and NAADP released by UV-photolysis. Whole-cell calcium transients were measured with a photomultiplier system. All data are given mean ± SEM with statistical significance considered as $p<0.05$.

Flash-photolysis of NAADP increased atrial calcium transients by 67 ± 15% ($p<0.05$, n=7), becoming significant 3min after photolysis and reaching a peak at 12min. This increase was prevented by 1μM patch-applied Ned-19.

In field-stimulated atrial myocytes, bafilomycin (100nM) significantly reduced cellular calcium transients (by 19 ± 5%, n=6) and inhibited enhancement of the calcium transient by isoprenaline (3nM), reducing it from a 63 ± 8% (n=9) to a 36 ± 9% (n=6) increase ($p<0.05$).

These data support the two-pool model for NAADP actions in cardiac atrial myocytes, suggest the NAADP system is constitutively active and indicate a physiological role in beta-adrenergic signalling.

1)Churchill and Galione EMBOJ (2001) 20: 2666-2671.
2)Macgregor et al. Cell Calcium (2007) 41:537-546.
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Pharmacological Evidence Suggests Acetate and Pyruvate Modulate Myocyte Systolic and Diastolic Function by Effects on Mitochondrial Ca^{2+} Handling

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We have recently demonstrated that there may be a direct link between energy substrate utilization and contractile function. Mice that have increased cardiac lipid storage and oxidation as a result of fatty acid transport protein 1 overexpression in the heart exhibit cellular diastolic dysfunction and acute exposure of myocytes to the long chain fatty acid palmitate attenuates contractility by disrupting E-C coupling. Glucose and long chain fatty acids are typical energy substrates that support cardiac function; however, cardiac myocytes can oxidize a variety of substrates for energy generation. For example, the short chain fatty acid, acetate, can be oxidized in preference to glucose in some circumstances. In the present study, we explore the effects of acute exposure to acetate or pyruvate on contractile function of isolated mouse ventricular myocytes. Acute exposure of myocytes to Tyrode solution supplemented with 10 mM sodium acetate causes a marked, but transient, decrease in sarcomere shortening (1.49 ± 0.20% vs. 5.58 ± 0.49% in control) and a significant increase in diastolic sarcomere length (1.77 ± 0.01 μm vs. 1.81 ± 0.01 μm in control) that persists in the continued presence of acetate for up to 60 minutes. Pyruvate (the major mitochondrial substrate), and dichloroacetate (indirectly stimulates mitochondrial pyruvate oxidation) cause similar effects. Interestingly, pretreatment of cells with the mitochondrial Ca^{2+} uptake blocker, Ru-360 (10 μM), markedly suppressed the effect of acetate on both myocyte contractile amplitude and diastolic sarcomere length. Taken together, the data suggest that 1) acute exposure to acetate or pyruvate can modulate both systolic and diastolic cardiac function and that 2) mitochondrial Ca^{2+} uptake seems to be a key mediator of this effect.